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ORIGINAL PAPER

Rapid Fluorescence Spectroscopic Characterization of Salivary DNA of Normal Subjects and OSCC Patients Using Ethidium Bromide

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Abstract Recently, deoxyribonucleic acid (DNA) based biomarker(s) detection has been employed for cancer diagnosis. Earlier reports have suggested the presence of more DNA in the saliva of oral squamous cell carcinoma (OSCC) than normal by electrophoresis technique. Based on these, steady state and excited state kinetics of salivary DNA has been performed with 27 normal subjects and 67 OSCC patients saliva using ethidium bromide as a probe to look for the possibility in discrimination between them. On statistical analysis the sensitivity and specificity of 88.9 and 94.0 % has been achieved from the fluorescence emission spectra and 88.9 and 92.5 % with that of fluorescence excitation.

Keywords Saliva mass screening · Salivary DNA · Ethidium bromide · Fluorescence steady and excited state kinetics · Simple and rapid technique

Introduction

Cancer is one of the most common dreaded diseases in the world. Among the various cancers, oral squamous cell carcinoma (OSCC) has been ranked number sixth [1, 2]. In the Asian continent, India in particular, the incident rate of oral cancer is predominant and has been ranked number one among males and third among females [3]. Histopathological discrimination of disease has been considered as the golden standard method, however, as the patients are not reporting to the physician at the early stage of the disease, due to lack of symptoms, the mortality rate has been raised up to 45 % for the last 5 years [4]. Early diagnosis of oral cancer may improve the life span of the patients due to treatment outcome [5]. Hence, there is a need for a novel rapid method for the early diagnosis of cancer. Any alteration in deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins and metabolites at molecular level are attributed to the onset of the disease and hence for the case of cancer also. In the case of cancer excess metabolic end products have been released due to the uncontrolled proliferation. These metabolites may have their impression in the body bio-fluids viz., blood [6, 7]; urine [8], saliva [7, 9–13]. In this context, diagnosis of disease at molecular level using biomarkers present in bio fluids have shown encouraging results. Among the various bio fluids, saliva was easily accessible and informative and hence, it has been considered for analyzing any changes at proteomics, genomics and metabolomics levels [10–13]. Nowadays, diagnosis of disease based on the characterization of DNA and RNA level has been considered as one of the highly sensitive and effective methods [10, 11]. Although, isolation and detection of DNA present in saliva by electrophoretic technique has been the most common and an accurate method, it was tedious and time consuming. In electrophoretic technique, ethidium bromide (EB) has been added as an intercalating cationic fluorescent dye, which covalently binds with the DNA helix and give fluorescence in the region where DNA present, under the illumination of ultraviolet light. In this regard, many have studied the interaction of EB with DNA by steady [14–17] and

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excited state kinetics [17, 18] to understand the nature of binding, binding site and the photophysical characteristics of the probe EB with DNA. Previously, it has been reported that, the fluorescence intensity of EB bounded DNA complex were comparatively higher than that of free EB. The increase in the intensity of EB-DNA complexes were attributed to the possibility of proton transfer to the base pair, which may increase the quantum yield of EB [15]. However, to the best of our knowledge there was no literature available in the characterization of DNA by fluorescence spectroscopy in disease discrimination. Many have discriminated cancer, based on native fluorescence spectroscopy, with the aid of fluorophores that are present in the tissues and biofluids [6, 8, 12]. Though DNA is present in the biofluids, it was not detectable by fluorescence spectroscopy due to its low quantum yield. Hence, in this present study, first of its kind, the good old fluorescence technique has been exploited with EB as a probe to characterize the salivary DNA of normal subjects and oral cancer. In the present study, the photophysical characteristics of EB in saliva has been characterized using fluorescence emission and excitation spectroscopy. The time resolved fluorescence spectroscopic characterizations has also been carried out in understanding the binding characteristics of ethidium bromide with salivary DNA. Further attempts have also been made to discriminate the saliva of cancer patients from that of normal subjects using the above techniques.

Materials and Methods

Study Group

For the present study, 67 patients with oral malignancy of age group of 25–80 (56±18) and 27 normal subjects with age group of 25–57 (36±15), of both male and female, were considered for the fluorescence spectroscopic analysis of saliva, after getting the ethical clearance from Health and Family Welfare Department, Government of Tamil Nadu, India. (Ref. no. 47846/ E2 /2012-1) and the patients consent were also obtained while collecting saliva samples. The control group were of non-drinking, non-smoking habits and were also free from the history of malignancy, immunodeficiency, auto immune disorder, and hepatitis (or) HIV infection. The oral cancer patients were histopathologically confirmed as oral squamous cell carcinoma (OSCC) at Government Arignar Anna Cancer Hospital, Kanchipuram, Tamilnadu, INDIA.

Collection of Saliva

Whole saliva was collected from both clinically confirmed normal subjects and cancer patients under resting conditions. One hour prior to the collection of saliva the patients were advised to stop any form of oral intake [12, 13]. The individuals was asked to gargle the oral cavity with clean water. After 15 min, the secreted saliva was collected in a sterile container. The samples thus collected were maintained at 4 °C and the entire spectroscopic analysis were made within 3 h from the time of collection, after thawing the samples to room temperature. We have eliminated the OSCC patients with visually larger tumor and open wound cases for this study.

Ethidium Bromide (EB)

EB was purchased from Sigma Aldrich co. USA, product code E7637 and the ethidium bromide stock solution was prepared with the phosphate buffer saline (PBS) of pH 7.4.

Calf Thymus DNA

Calf thymus DNA was purchased from Sigma Aldrich co. USA, product code-D8515 and the standard solution was prepared using PBS of pH 7.4.

Fluorescence Spectroscopic Characterization

The fluorescence spectroscopic characterization was carried out using spectroflurometer of model Fluoromax-2, SPEX, New Jersey, USA with150 W Ozone free Xenon arc lamp as the excitation source, and a photomultiplier tube (R928P; Hamamatzu, Shizuoka-Ken, Japan) as the detector. Excitation and emission slit width were fixed as 5 nm. The acquisition was made, with 1 nm spectral interval and 0.1 s integration time.

The steady state fluorescence emission spectra were measured from 500 nm to 750 nm at 480 nm excitation. The fluorescence excitation spectra were measured from 250 nm to 580 nm for 600 nm emission with 12.5 μmol (μM) of EB in 1 ml of saliva of normal subjects and OSCC patients at room temperature.

Fluorescence Excited State Kinetics

Lifetime measurements were made using Time Correlated Single Photon Counting (TCSPC) System on Fluorolog-3 (HORIBA Jobin Yvon, INC, NJ) by exciting the sample using 460 nm Nano LED (Pulse Width: >1 ns), with a fast response red sensitive PMT(R928P, Hamamatsu Photonics, Shizuoka-Ken, Japan) detector. The instrument response function was obtained using the Rayleigh scatter of ludox-40 (40 weight percentage suspension in water; Sigma-Aldrich) at 460 nm excitation. Decay analysis software (DAS6 v6.0, Horiba) was used to extract the lifetime components and their amplitudes. The goodness of fit were judged by chi-square values, Durbin-Watson parameters, as well as the residuals, and autocorrelation functions.

Multiple Linear Discriminant Analysis

The multiple linear discriminant analysis was performed, as detailed in the earlier reports using SPSS[6] across two groups of, saliva from 27 normal subjects and 67 histopathologically confirmed OSCC patients. The discriminant analysis used a partial F -test by sequentially incorporating the integrated area into a Fisher linear discriminant function.

Results

Fluorescence Emission Spectrum for 480 nm Excitation

To study the intercalation of EB with salivary DNA, fluorescence emission spectra of both normal subjects and OSCC patients are recorded after adding 12.5 μM EB in 1 ml of saliva and exciting them at 480 nm. For comparison, the fluorescence emission spectra of free EB in PBS are also recorded under similar experimental conditions and are shown in Fig. 1. From the Fig. 1, it is observed that the overall emission characteristics of EB in saliva of OSCC is higher than that of EB in normal saliva and PBS, in the order of EB in OSCC saliva > in normal saliva > in PBS. To confirm that the enhancement in the fluorescence intensity of EB in saliva is due to its interaction with DNA, fluorescence emission spectra of EB with commercially available DNA and free EB in PBS are also measured and are shown in the inset of Fig. 1. From the Fig. 1 and its inset, it is clear that the enhanced emission of EB is due to the intercalation of EB with DNA.

Figure 2 shows the normalised fluorescence emission spectra of EB in PBS, in normal saliva and in OSCC saliva. The insert in the Fig. 2 explains the normalised fluorescence emission of chemical EB-DNA complex and EB in PBS. A blue shift in the fluorescence emission spectra of EB, after intercalation with DNA is evident from the Fig. 2 and its insert.

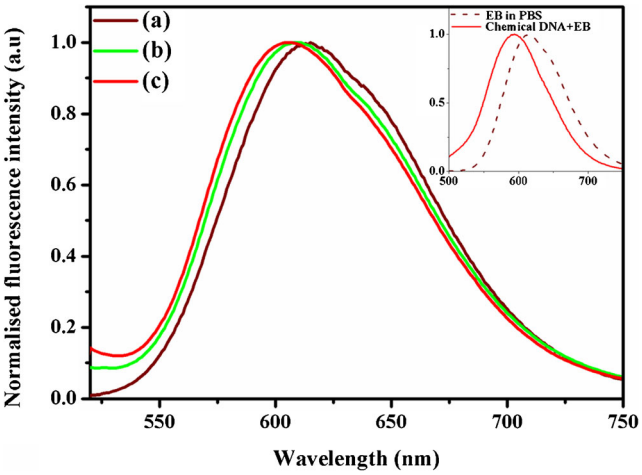

Fig. 2 Normalised average fluorescence emission spectrum of EB in **a** PBS **b** saliva of normal subjects and **c** saliva of OSCC patients. The *dotted line* in the inset denotes that of EB in PBS and the *solid line* represents the EB in chemical DNA, at 480 nm excitation

Fluorescence Excitation Spectral Characteristics of Saliva at Normal Subjects and OSCC Patient for 600 nm Emission

The fluorescence excitation spectra for 12.5 μM of EB in 1 ml of saliva of normal subjects, in OSCC patients and in PBS are measured for 600 nm emission under similar conditions and are shown in the Fig. 3. From the Fig. 3, it is observed that the overall fluorescence excitation intensity of EB in saliva are in

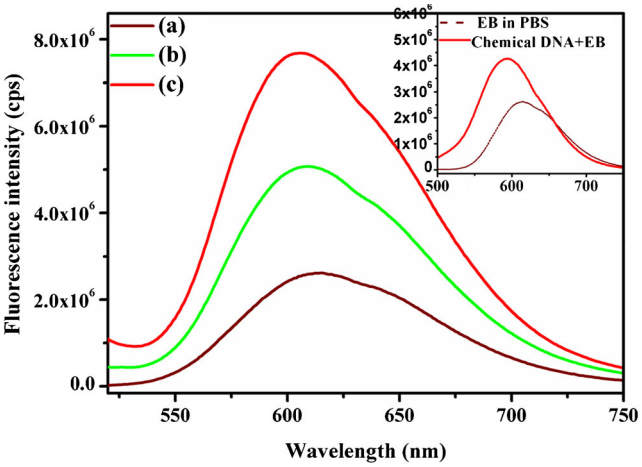

Fig. 1 Averaged fluorescence emission spectrum for 12.5 μM of EB in 1 ml of **a** PBS **b** saliva of normal subjects and **c** saliva of OSCC patients. In the inset figure, the *dotted line* depicts the emission of EB in PBS and the *solid line* represents that of EB in commercially available chemical DNA, at 480 nm excitation

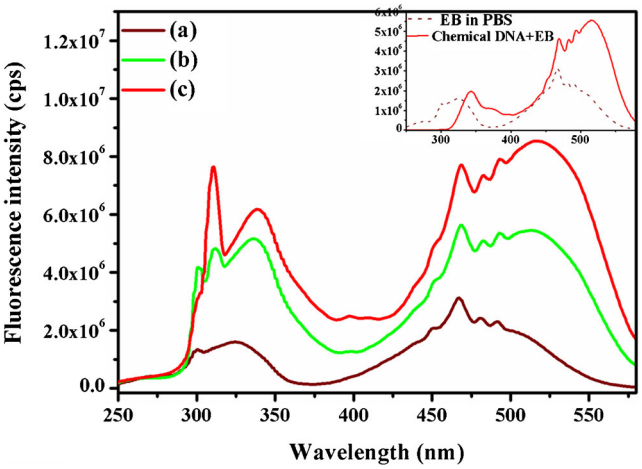

Fig. 3 Averaged fluorescence excitation spectra for 12.5 μM of EB in 1 ml of **a** PBS, **b** saliva of normal subjects and **c** saliva of OSCC patients. In the inset figure, the *dotted line* depicts the excitation spectra of EB in PBS and the *solid line* represents that of EB in commercially available chemical DNA for 600 nm emission

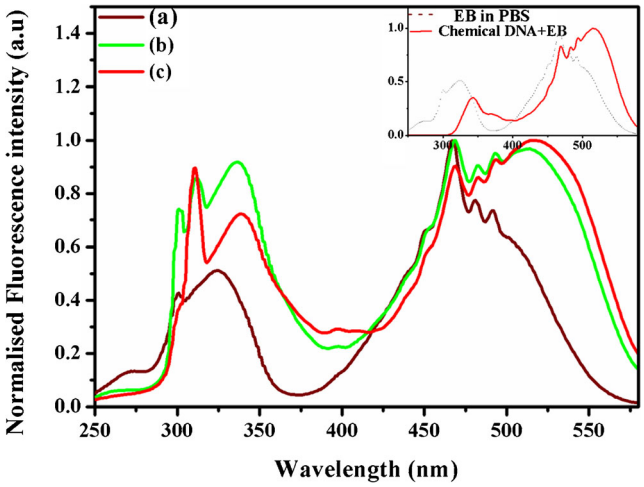

Fig. 4 Normalised average fluorescence excitation spectra of EB in **a** PBS, **b** saliva of normal subjects and **c** saliva of OSCC patients. The *dotted line* in the inset denotes that of EB in PBS and the *solid line* represent the same concentration of EB in chemical DNA for 600 nm emissions

the order of EB in OSCC saliva > in normal saliva > in PBS. To verify and confirm the observed fluorescence emission peak around 600 nm are due to EB, the fluorescence excitation spectra are exploited as an indirect tool. Further to confirm the enhanced intensity is due to intercalation with DNA, fluorescence excitation spectra of EB in commercially available DNA are also measured and are shown as an inset in the Fig. 3. The overall fluorescence excitation intensity for DNA-EB complex is higher than that of EB in PBS, the same as observed in saliva.

Figure 4 shows the normalised fluorescence excitation spectra of EB in PBS, in normal saliva and in OSCC saliva and the inset depicts that of the chemical DNA-EB complex and EB in PBS. A red shift is observed in the fluorescence excitation maxima for chemical DNA-EB complex when compared to free EB, and the same is also observed between EB in saliva and the EB in PBS. The shift is considerably more for EB in OSCC than that in normal. This may be due to the presence of more DNA in OSCC saliva than that in normal saliva.

Excited State Kinetics of Saliva at 600 nm

The excited state kinetics of EB in PBS, in saliva of normal subjects, in OSCC patients and in chemical DNA for 600 nm emission maxima are performed for comparison. The decay curves are analysed using non-linear least square fitting method and it is found that the fit for free EB in PBS is mono exponential with the lifetime of 1.6 ns whereas, the EB in chemical DNA, normal and OSCC saliva resulted in tri-exponantial fit with least chi square values lesser than 1.3 and the data are shown in Table 1. In order to know whether the change in the decay characteristics of EB is due to the binding of salivary protein, the decay characteristics of EB with bovine serum albumin (BSA) are also performed. The resulted bi exponential fit with the lifetime values of 2.3 and 1.5 ns with the corresponding amplitude contribution 20.3 and 79.7 % respectively, and the absence of longer life time confirms the refrain of salivary protein contribution.

Results of Statistical Analysis

To estimate the statistical significance of the observed data, the integrated area of the fluorescence emission and fluorescence excitation spectra are subjected for multiple linear discriminant analysis.

Discriminant Analysis

Multiple linear discriminant analysis has been performed with the 67 oral cancer and 27 normal samples. Figures 5 and 6 shows the scatter plot for the discriminant score of the integrated area of the fluorescence emission spectra and the fluorescence excitation spectra respectively. The group centroids

Table 1 Mean amplitude and lifetime values of 12.5 μmol concentration of EB in 1 ml of PBS, chemical DNA, saliva of normal subjects, OSCC patients and BSA for the emission decay at 600 nm along with the P value of Normal Vs OSCC

Sample	Slow component		Intermediate component		Fast component	
	A 1(%)	T1 (ns)	A 2(%)	T2 (ns)	A 3(%)	T3 (ns)
EB in PBS					100	1.6
BSA+EB		20.3	2.3	79.7	1.5	
Chemical DNA+EB	41.9	19.6	36.7	10.7	21.3	1.6
Normal	22.3±6.2	20±1.8	7.7±4	5.2±2	73.4±7	1.6±0.34
OSCC	58.7±9.4	20.7±0.77	9.9±5.2	6.15±1.8	31.4±9.3	1.5±0.1
P value (Normal Vs OSCC)	0.000	0.01	0.06	0.04	0.000	0.385

A (%) - Amplitude in percentage

T (ns) - Lifetime in nanoseconds

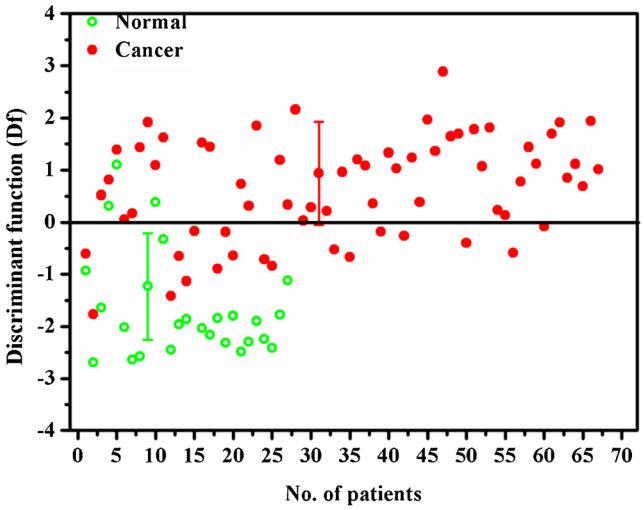

Fig. 5 *Scatter plot* showing the discrimination of EB in saliva of normal subjects and OSCC patients based on the fluorescence emission at 480 nm excitation

with the standard deviations are shown in both the figures. The classification based on the discriminant analysis for the fluorescence emission and the fluorescence excitations are given in Tables 2 and 3 respectively.

Discussion

Despite the technological advancements in the therapeutic modalities, there is a raise in the oral cancer mortality and morbidity rate, which may be due to the late stage diagnosis of oral cancer [1, 2]. To overcome the limitations of the prevailing visual examination and the subjective hystopathological examination, there is a need for new diagnostic methods. In this juncture, DNA based biomarker(s) are considered in designing new diagnostic modalities. Cancer cells may have abnormally elevated level of oxidative enzymes [19, 20] and there may be a possibility of formation of free radicals, such as reactive oxygen species (ROS) like superoxide radicals (O_2*), Hydroxyl radicals (OH*), Hydrogen peroxide (H_2O_2) and reactive nitrosamines (RNS) like nitric oxide (NO), nitrites (NO_2) and nitrates (NO_3), which may be involved in the DNA damage [10, 19]. Earlier Bahar et al. [10], have reported the 65 % of oxidised DNA in the saliva of OSCC when compared to that of control groups by electrophoretic technique. Currently, much attention has been gathered in the evaluation of DNA in saliva of OSCC [10, 11]. DNA isolation and detection by means of electrophoretic technique were widely practiced due to its sensitivity and accuracy. However minimum quantities of DNA may not be detectable, and hence, the DNA sequence were amplified by means of either polymerase chain

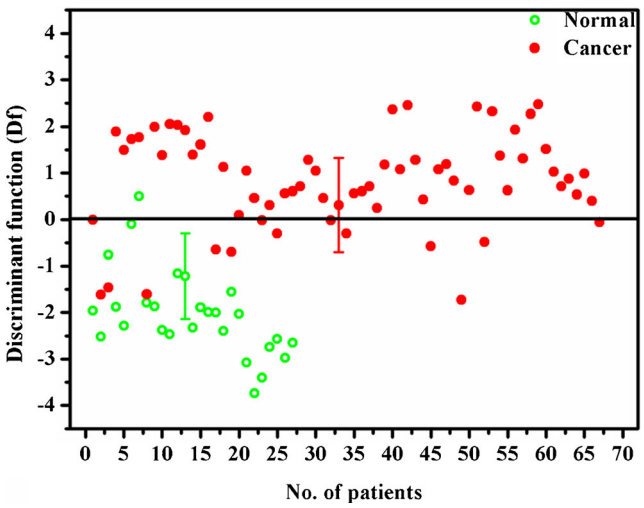

Fig. 6 *Scatter plot* showing the discrimination based on the fluorescence excitation spectrum for 600 nm emission of EB in saliva of normal subjects and OSCC patients

Table 2 Classification results of discriminant analysis for different groups based fluorescence emission at 480 nm excitation

		Group	Predicted group membership		Total
			1.00	2.00	
Original	Count	1.00	23	4	27
		2.00	4	63	67
	%	1.00	88.9	11.1	100.0
		2.00	6.0	94.0	100.0
Cross-validated	Count	1.00	24	3	27
		2.00	4	63	67
	%	1.00	88.9	11.1	100.0
		2.00	6.0	94.0	100.0

Specificity/Sensitivity values of the corresponding groups are expressed in percentage

Table 3 Classification results of discriminant analysis for different groups based fluorescence excitation spectra for 600 nm emission

		Group	Predicted Group Membership		Total
			1.00	2.00	
Original	Count	1.00	24	3	27
		2.00	4	63	67
	%	1.00	88.9	11.1	100.0
		2.00	6.0	94.0	100.0
Cross-validated	Count	1.00	24	3	27
		2.00	5	62	67
	%	1.00	88.9	11.1	100.0
		2.00	7.5	92.5	100.0

Specificity/Sensitivity values of the corresponding groups are expressed in percentage

reaction (PCR) or micro array analysis method. In all these methods fluorescence from EB were widely employed as a probe for detecting DNA and RNA. From the earlier literature we can infer that, small fragment of DNA was sufficient to account for any change in the emission intensity [14–17], wavelength shift [16, 21], fluorescence polarization, and fluorescence lifetime [18] with EB as a probe. EB with nucleic acids as the basic concept, we have made a pilot study in discriminating the saliva of normal subjects from that of OSCC patients using the steady and time resolved fluorescence spectroscopy. Attempts were also made to analyse the spectra of fluorescence emission, fluorescence excitation and time resolved fluorescence to check the variation in the photophysical characteristics of normal and OSCC cases. Statistical analysis were also made to test the feasibility to employ this simple and rapid technique for mass screening of oral cancer using salivary DNA.

From the Fig. 1, it was observed that the overall emission intensity at 480 nm excitation were in the order of EB in OSCC > in normal > in PBS. The inset in Fig.1 confirm the increase in the fluorescence intensity of saliva were due to the interaction of EB with salivary DNA. Figure 2 and the inset show the emission spectra of free EB and EB-DNA complex. From the Fig. 2 and its inset, a clear blue shift was observed for EB in saliva and EB in DNA when compared to that of free EB. The shift for EB in OSCC, normal saliva and chemical DNA were about 12, 7 and 20 nm respectively. The enhancement in the overall fluorescence emission and blue shift may be attributed to the following reasons: It was reported that the fluorescence emission of the EB-DNA complex were based on the binding efficiency between them [15–17, 21]. At 480 nm excitation EB in PBS may get excited and water in PBS may get splitted into H^+ and OH^-, due to the resonance energy transfer from the highly excited EB [22]. On the other hand, in the case of EB-DNA complex the energy may be transferred to the base pair of the DNA helix due to the covalent binding and electrostatic force between phosphate group of DNA and that of nitrogen group in EB, which were characterised by more fluorescence quantum efficiency compared to that of EB in PBS.[15]The present study also confirms the same.

In the case of fluorescence excitation spectra for 600 nm emission, an increase in the intensity of fluorescence emission was observed (Fig. 3), but with a red shift for EB-DNA complex when compared to free EB (Fig. 4) which were well correlated with the previous literatures, [16, 21] conforming intercalation of EB with DNA. The enhancement in the overall fluorescence intensity of both the emission and excitation in OSCC cases may be due to the availability of more DNA than normal subjects. This was further confirmed by the measurement of the time resolved fluorescence spectroscopical characterization of free EB and EB-DNA complex. EB in PBS was mono exponential whereas EB-DNA complex exhibited tri-exponantial fit with slow (τ_1), intermeadiate (τ_2) and fast (τ_3) components, respective amplitudes as shown in Table 1. The possible reason for the different lifetimes for EB-DNA complex may be due to the presence of two ring structure in EB, one phenanthridine ring which may be involved in DNA intercalation (covalent bonding) between the helix, which stays much more longer time in the excited state may account for longer lifetime, the other phenyl ring which may freely rotate in the surface of the DNA helix may be responsible for the intermediatory lifetime [18] and the shorter lifetime may be due to the presence of free dye [17, 18]. Decay kinetics of BSA-EB for 600 nm emission, which resulted in bi-exponantial fit and devoid of longer lifetime confirmed the nonparticipation of salivary protein. The chemical DNA-EB complex results were well correlated with that of saliva–EB data, indicating clearly that the change in the fluorescence intensity and fluorescence lifetime may due to the interaction of EB with DNA. The increase in the amplitudes may be attributed to the presence of more DNA in OSCC saliva than normal.

The observed spectral difference were analysed for statistical significance. The discriminant D, differenciated the fluorescence emission spectra of OSCC patients from normal subjects with 88.9 % specificity and 94.0 % sensitivity, as shown in (Table 2). On the other hand the fluorescence excitation spectrum discriminated the same with 88.9 % specificity and 92.5 % sensitivity, as shown in (Table 3). Based on the statistical significance the present technique may be exploited to discriminate saliva of oral cancer patients from that of normal subjects. In conclusion to the best of our knowledge, no reports were available in the characterization of oral cancer patient's saliva from normal subjects using fluorescence technique. For that reason, first of its kind, the fluorescence spectroscopic characterization of saliva after the intercalation with EB revealed statistically significant variation in the spectral signature of normal and OSCC cases. Hence, this technique can override the time consuming electrophoresis technique and so can be employed for rapid preliminary mass screening.

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References

1. The international statistical classification of diseases and related health problems (1992) Geneva. World Health Organization 10th Ed
2. Cancer Facts and Figures (2007) Atlanta. American Cancer Society
3. Ravindran R, Deepa MG (2012) Salivary tumour markers in oral cancer: brief review. Oral Maxillofac Pathol J 2:238–244

4. Markopoulos AK, Michailidou EZ, Tzimagiorgis G (2010) Salivary markers for oral cancer detection. Open Dent J 4:172–178
5. Coelho KR (2012) Challenges of the oral cancer burden in India. J Cancer Epidemol Article ID 701932: 1–17. doi: 10.1155/2012/701932
6. Madhuri S, Vengadesan N, Aruna P, Koteeswaran D, Venkatesan P, Ganesan S (2003) Native fluorescence spectroscopy of blood plasma in the characterization of oral malignancy. Photochem Photobiol 78(2):197–204
7. Hansen TVO, Simonsen MK, Nielsen FC (2007) Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. Cancer Epidemiol Biomarkers Prev 16:2072–2076
8. Rajasekaran R, Aruna PR, Koteeswaran D, Bharanidharan G, Baludavid M, Ganesan S (2014) Steady-state and time-resolved fluorescence spectroscopic characterization of urine of healthy subjects and cervical cancer patients. J Biomed Opt 19(3):037003
9. Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, Gavish M, Nagler RM (2009) Salivary analysis of oral cancer biomarkers. Br J Cancer 101(7):1194–1198
10. Bahar G, Feinmesser R, Shiptzer T, Popovtzer A, Nagler RM (2007) Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer 109(1): 54–59
11. Viet CT, Schmidit BL (2008) Methylation array analysis of preoperative and postoperative saliva DNA in oral cancer patients. Cancer Epidemiol Biomarkers Prev 17:3603–3611
12. Yuvaraj M, Udayakumar K, Jayanth V, Prakasa Rao A, Bharanidharan G, Koteeswaran D, Munusamy BD, Krishna CM, Ganesan S (2014) Fluorescence spectroscopic characterization of salivary metabolites of oral cancer patients. J Photochem Photobiol B 130:153–160
13. Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita (2010) Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. Metabolomics 6:78–95
14. Byrne CD, de Mello AJ (1998) Photophysics of ethidium bromide complexed to ct-DNA: a maximum entropy study. Biophys Chem 70: 173–184
15. Vardevanyan PO, Antony AP, Parsadanyan MA, Davtyan HG, Karapetyan AT (2003) The binding of ethidium bromide with DNA: interaction with single-and double-stranded structures. Exp Mol Med 35(6):527–533
16. Cosa G, Focsaneanu KS, McLean JRN, Mc Namee JP, Scaiano JC (2001) Photophysical properties of fluorescent DNA-dyes bound to single- and double-stranded DNA in aqueous buffered solution. Photochem Photobiol 73(6):585–599
17. Olmsted J III, Kearns RD (1977) Mechanism of ethidium bromide fluorescence enhancement on binding to nucleic acids. Biochemistry 16(16):3647–3654
18. Wahl P, Paoletti J, Le Pecq JB (1970) Decay of fluorescence emission anisotropy of the ethidium bromide-DNA complex evidence for an internal motion in DNA. Proc Natl Acad Sci U S A 65(2):417–421
19. Salem MMAEL, Shalbaf M, Gibbons NCG, Chavan B, Thornton MJ, Schallreuter KU (2009) Enhanced DNA binding capacity on up-regulated epidermal wild-type p53 in vitiligo by H_2O_2-mediated oxidation: a possible repair mechanism for DNA damage. FASEB J Res Commun 23:3790–3807
20. Modica-Napolitano JS, Singh KK (2002) Mitochondria as targets for detection and treatment of cancer. Expert Rev Mol Med 4(9):1–9
21. Dragan AI, Bishop ES, Casas-Finet RJ, Schenerman MA, Geddes CD (2009) Metal-enhanced ethidium bromide emission: application to dsDNA detection. Chem Phys Lett 480:296–299
22. Keskinova E, Angelov D, Angelova SV, Shaqiri Z, Veseli A (1998) Effects of high-intensity UV radiation on isolated and DNA-intercalated ethidium bromide. Fizika A 7:17–26